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Gene-specific facial dysmorphism in Axenfeld-Rieger syndrome caused by *FOXC1* and *PITX2* variants

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Abstract

Axenfeld-Rieger syndrome is a genetic condition characterized by ocular and systemic features and is most commonly caused by variants in the *FOXC1* or *PITX2* genes. Facial dysmorphism is part of the syndrome but the differences between both genes have never been systematically assessed. Here, 11 facial traits commonly reported in Axenfeld-Rieger syndrome were assessed by five clinical geneticists blinded to the molecular diagnosis. Individuals were drawn from the Australian and New Zealand Registry of Advanced Glaucoma in Australia or recruited through the Genetic and Ophthalmology Unit of l'Azienda Socio-Sanitaria Territoriale Grande Ospedale Metropolitano Niguarda in Italy. Thirty-four individuals from 18 families were included. *FOXC1* variants were present in 64.7% of individuals and *PITX2* variants in 35.3% of individuals. A thin upper lip (55.9%) and a prominent forehead (41.2%) were common facial features shared between both genes. Hypertelorism/telecanthus (81.8% vs 25.0%, $p = 0.002$) and low-set ears (31.8% vs 0.0%, $p = 0.036$) were significantly more prevalent in individuals with *FOXC1* variants compared with *PITX2* variants. These findings may assist clinicians in reaching correct clinical and molecular diagnoses, and providing appropriate genetic counseling.

KEYWORDS
Axenfeld-Rieger syndrome, facial dysmorphism, *FOXC1*, *PITX2*

1 | INTRODUCTION

Axenfeld-Rieger syndrome (ARS) is an autosomal dominant condition characterized by ocular and systemic features. Ocular features consist of the anterior segment dysgenesis spectrum characterized by developmental abnormalities of the anterior segment of the eye. They include abnormal angle tissue contributing to a high risk of secondary glaucoma, hypoplastic iris, additional pupillary opening (pseudopolycoria), displaced pupil (corectopia), anteriorly displaced Schwalbe's line (posterior embryotoxon), and/or peripheral anterior synechiae (Alward, 2000; Shields et al., 1985). The most commonly reported systemic features include facial dysmorphism, dental and umbilical anomalies, cardiac defects, and hearing impairment (Alward, 2000; Fitch & Kaback, 1978). Deleterious sequence variants and copy number variation of the *FOXC1*

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(6p25.3, MIM 601090) (Mears et al., 1998; Nishimura et al., 1998) and *PITX2* (4q25, MIM 601542) (Semina et al., 1996) genes have been implicated in ARS, as well as another locus (RIEG2 at 13q14) (Phillips et al., 1996). *FOXC1* and *PITX2* variants explain ~40% of affected individuals (D'Haene et al., 2011; Reis et al., 2012).

Although the ocular manifestations of the condition associated with variation of both genes are similar, the systemic features often vary: individuals with *FOXC1* variants are more likely to present with an isolated ocular phenotype or display a range of systemic features among which cardiac defects, hearing loss, and growth delay are the most common. In comparison, *PITX2* variants are strongly associated with the ocular phenotype in combination with dental anomalies (microdontia, hypodontia) and umbilical defects (periumbilical skin, umbilical hernia) (D'Haene et al., 2011; Reis et al., 2012; Souzeau et al., 2017; Strungaru et al., 2007). Minor facial dysmorphism has been identified as part of the ARS spectrum since the initial reports of affected individuals, with characteristic facial features including maxillary hypoplasia with flattening of the midface, hypertelorism, a broad flat nasal root, and a thin upper lip (Alkemade, 1969; Rieger, 1941).

Assessment of dysmorphic features, including facial dysmorphism, plays a major role in the evaluation of genetic syndromes to reach a clinical diagnosis. As such, a number of genetic syndromes have well characterized and recognizable facial features. Although previous studies have reported on some facial features in cohorts of individuals with ARS (Reis et al., 2012; Weisschuh et al., 2006), no study has systematically assessed the prevalence of different facial traits. In this study, we compared facial dysmorphism between individuals with ARS due to *FOXC1* or *PITX2* variants to assess gene-specific differences.

TABLE 1 Definition of the facial traits

Prominent supraorbital ridges	Greater than average forward and/or lateral protrusion of the supraorbital portion of the frontal bones
High arched eyebrows	Increased height of the central portion of the eyebrow, forming a crescent, semicircular, or inverted U shape
Prominent forehead	Forward prominence of the entire forehead, due to protrusion of the frontal bone
Hypertelorism	Increased distance between the orbits, where the inner canthal distance, the outer canthal distance and interpupillary distance are increased
Telecanthus	Increased distance between the inner canthi with normal interpupillary distance
Broad flat nasal bridge	Increased breadth of the nasal bridge (and with it, the nasal root)
Maxillary hypoplasia	Abnormally small dimension of the maxilla, usually creating a malocclusion or malalignment between the upper and lower teeth or resulting in a deficient amount of projection of the base of the nose and lower midface region
Protruding lower lip	Abnormal configuration of the lower lip such that it is turned outward (i.e., everted), with the inner aspect of the lower lip vermilion (normally opposing the teeth) being visible in a frontal view
Thin upper lip	Reduced width of the skin of the vermilion border region of the upper lip
Short philtrum	Apparently decreased distance between nasal base and midline upper lip vermilion border
Low-set ears	Upper insertion of the ear to the scalp below an imaginary horizontal line drawn between the inner canthi of the eye and extending posteriorly to the ear

2 | METHODS

2.1 | Subjects

The study was conducted in accordance with the revised Declaration of Helsinki. Ethics approval was obtained from the Southern Adelaide Clinical Human Research Ethics Committee (Australia), l'Azienda Socio-Sanitaria Territoriale Grande Ospedale Metropolitano Niguarda (Italy) and the Friedrich-Alexander University of Erlangen-Nuremberg Ethics Committee (Germany). Individuals were drawn from the Australian and New Zealand Registry of Advanced Glaucoma (ANZRAG) (Souzeau et al., 2012) or recruited through the Genetic and Ophthalmology Unit of l'Azienda Socio-Sanitaria Territoriale Grande Ospedale Metropolitano Niguarda. Informed consent was obtained from all participants or their guardians. Ethnicity was self-reported. Individuals with ASD were tested for variants in the *FOXC1* and *PITX2* genes by sequencing and MLPA as previously described (Siggs et al., 2019; Souzeau et al., 2017).

2.2 | Facial dysmorphology

Clinical photographs (frontal and lateral views of the face) of individuals with ARS were obtained at the time of appointment or provided directly by the participants. The clinical photographs were reviewed independently by a panel of 5 clinical geneticists experienced in dysmorphology, blinded to the molecular diagnosis of the participants. The assessment of the facial traits was subjective and therefore the presence of the facial traits was considered to be dependent on the consensus of at least 4 of the 5 geneticists. The presence or absence of 11 facial traits commonly reported in ARS were recorded for each participant including: prominent supraorbital ridges, high arched eyebrows, prominent forehead, hypertelorism, telecanthus, broad flat nasal bridge, maxillary hypoplasia, protruding lower lip, thin upper lip, short philtrum and low-set ears. The facial traits were defined according to the Human Phenotype Ontology (Köhler et al., 2019) and the Bedside Dysmorphologist (Reardon, 2015) (Table 1). Due to the subjective nature of the assessment, hypertelorism and telecanthus were combined together for analysis purposes.

2.3 | Face averaging

Images from individuals with *FOXC1* or *PITX2* variants were combined into a single consensus image using the dlib and OpenCV packages in

Python (https://github.com/leoneckert/facemash-workshop). Each image was subject to facial detection and landmark annotation, followed by scaling and alignment, with all images for each gene overlaid to create an "average" face image. Separate consensus images were generated for each gene for probands only (using the youngest photograph in each family) and for all individuals.

2.4 | Statistics

PASW Statistics, Rel. 18.0.1.2009 (SPSS Inc., Chicago, IL) was used for statistical analyses. Data are presented as mean ± *SD*. Fisher's exact test was used to assess differences in categorical data. Mann–Whitney *U* test and Median test were used to assess differences in mean and median respectively.

3 | RESULTS

Thirty-four individuals from 18 families were included. The mean age at recruitment was 29.6 ± 19.5 years and all individuals were Caucasian, apart from one who had a mixed Caucasian/Asian ethnicity (Individual 5). *FOXC1* variants were present in 64.7% (22/34) individuals or 72.2% (13/18) families and *PITX2* variants in 35.3% (12/34) individuals or 27.8% (5/18) families. There were no differences in sex or age between individuals with *FOXC1* vs *PITX2* variants (Table 2). The ocular and systemic features that support the findings of this study are available in Supplementary Table 1.

The prevalence of the different facial traits in individuals with *FOXC1* or *PITX2* variants is summarized in Table 3. The average number of facial traits for the whole cohort was 2.71 ± 1.40 (range 0–5). The most common features in the whole cohort seen in over a third of individuals were telecanthus/hypertelorism, (61.8%), a thin upper lip (55.9%), and a prominent forehead (41.2%).

Individuals with *FOXC1* variants had an average of 2.86 ± 1.52 facial features (range 0–5) compared with 2.42 ± 1.16 (range 0–4) for individuals with *PITX2* variants (p = 0.309). There was no differences in the number of facial features between individuals who had sequence variants (n = 2.81 ± 1.41) and those with copy number variations (n = 2.29 ± 1.38, p = 0.452). The most common traits in *FOXC1* were a telecanthus/hypertelorism (81.2%), a thin upper lip (45.5%) and a prominent forehead (36.4%). In comparison, the most common traits in *PITX2* were a thin upper lip (75.0%), a prominent forehead (50.0%) and prominent supraorbital ridges (33.3%). Hypertelorism/telecanthus and low-set ears were significantly more prevalent in individuals with *FOXC1* variants compared with *PITX2* variants. Although not significantly different, a thin upper lip was more common in individuals with *PITX2* (75.0%) compared to *FOXC1* (45.5%) and maxillary hypoplasia was only present among individuals with *FOXC1* variants (27.3%). Additionally, geneticists could comment on the presence of other potential facial traits and the presence of hooded eyelids or ptosis was suggested in some individuals. However, these traits cannot be formerly assessed on clinical photographs and would need to be assessed in person to investigate potential differences between both groups.

In order to reduce potential bias related to familial facial features unrelated to the *FOXC1* or *PITX2* variants, the assessed facial traits were further analyzed in probands only from 18 families, and was done using individuals with photographs taken at the youngest age within each family. The analysis included 13 individuals with *FOXC1* and 5 with *PITX2* variants. There were no differences in sex or age between individuals with *FOXC1* vs *PITX2* variants (Table 2). Hypertelorism/telecanthus was still significantly more prevalent in individuals with *FOXC1* variants compared with *PITX2* variants while low-set ears was not (Table 3).

In order to generate a consensus facial image for each genotype, face averaging was performed on photographs of individuals with *FOXC1* or *PITX2* variants (Figure 1). An increased distance between the medial orbital canthi (telecanthus) was subjectively apparent in average images of the *FOXC1* group, both using all individuals, and in probands alone.

Additionally, we assessed the prevalence of other systemic features in individuals with *FOXC1* or *PITX2* variants (Supplementary Table 2). Individuals with *FOXC1* variants were more likely to have no other systemic features compared with individuals with *PITX2* variants (p = 0.013). Dental and umbilical anomalies were more prevalent in individuals with *PITX2* variants compared with *FOXC1* variants (p < 0.001) whereas hearing loss was more prevalent in individuals with *FOXC1* variants compared with *PITX2* variants (p = 0.036).

4 | DISCUSSION

Facial dysmorphology, as part of a systematic medical examination and in combination with a complete family history, can assist clinicians

TABLE 2 Demographic details of the entire cohort and probands only

	All individuals				Probands			
	Total *N* (%)	*FOXC1 N* (%)	*PITX2 N* (%)	*p* value	Total *N* (%)	*FOXC1 N* (%)	*PITX2 N* (%)	*p* value
Sex (male)	16/34 (47.1)	11/22 (50.0)	5/12 (41.7)	0.729	11/18 (61.1)	9/13 (69.2)	2/5 (40.0)	0.326
Ethnicity (Caucasian)	33/34 (97.1)	21/22 (95.5)	12/12 (100.0)	1.000	17/18 (94.4)	12/13 (92.3)	5/5 (100.0)	1.000
Age when photographed (years)								
Mean	29.6 ± 19.5	27.3 ± 21.6	33.8 ± 14.6	0.231	19.9 ± 18.1	18.3 ± 20.0	24.2 ± 12.4	0.289
Median	32.0	24.5	36.0	0.720	11.5	7.0	22.0	0.294
Range	1-70	1-70	7-56		1-60	1-60	7-39	

TABLE 3 Prevalence of facial traits in all individuals and probands only with *FOXC1* or *PITX2* variants

	All individuals				Probands			
Facial trait	**Total *N* (%)**	***FOXC1* N (%)**	***PITX2* *N* (%)**	***p* value**	**Total *N* (%)**	***FOXC1* *N* (%)**	***PITX2* N (%)**	***p* value**
Prominent supraorbital ridges	10/34 (29.4)	6/22 (27.3)	4/12 (33.3)	0.714	6/18 (33.3)	5/13 (38.5)	1/5 (20.0)	0.615
High arched eyebrows	4/34 (11.8)	1/22 (4.5)	3/12 (25.0)	0.115	1/18 (5.6)	1/13 (7.7)	0/5 (0.0)	1.000
Prominent forehead	14/34 (41.2)	8/22 (36.4)	6/12 (50.0)	0.487	8/18 (44.4)	5/13 (38.5)	3/5 (60.0)	0.608
Hypertelorism/Telecanthus	21/34 (61.8)	18/22 (81.8)	3/12 (25.0)	0.002	12/18 (66.7)	12/13 (92.3)	0/5 (0.0)	0.001
Broad flat nasal bridge	3/34 (8.8)	3/22 (13.6)	0/12 (0.0)	0.537	2/18 (11.1)	2/13 (15.4)	0/5 (0.0)	1.000
Maxillary hypoplasia	6/34 (17.6)	6/22 (27.3)	0/12 (0.0)	0.069	4/18 (22.2)	4/13 (30.8)	0/5 (0.0)	0.278
Protruding lower lip	3/34 (8.8)	1/22 (4.5)	2/12 (16.7)	0.279	3/18 (16.7)	1/13 (7.7)	2/5 (40.0)	0.172
Thin upper lip	19/34 (55.9)	10/22 (45.5)	9/12 (75.0)	0.152	10/18 (55.6)	6/13 (46.2)	4/5 (80.0)	0.314
Short philtrum	4/34 (11.8)	2/22 (9.1)	2/12 (16.7)	0.602	3/18 (16.7)	2/13 (15.4)	1/5 (20.0)	1.000
Low-set ears	7/34 (20.6)	7/22 (31.8)	0/12 (0.0)	0.036	3/18 (16.7)	3/13 (23.1)	0/5 (0.0)	0.522

Note: Fisher's exact test was used to assess differences between both genes.

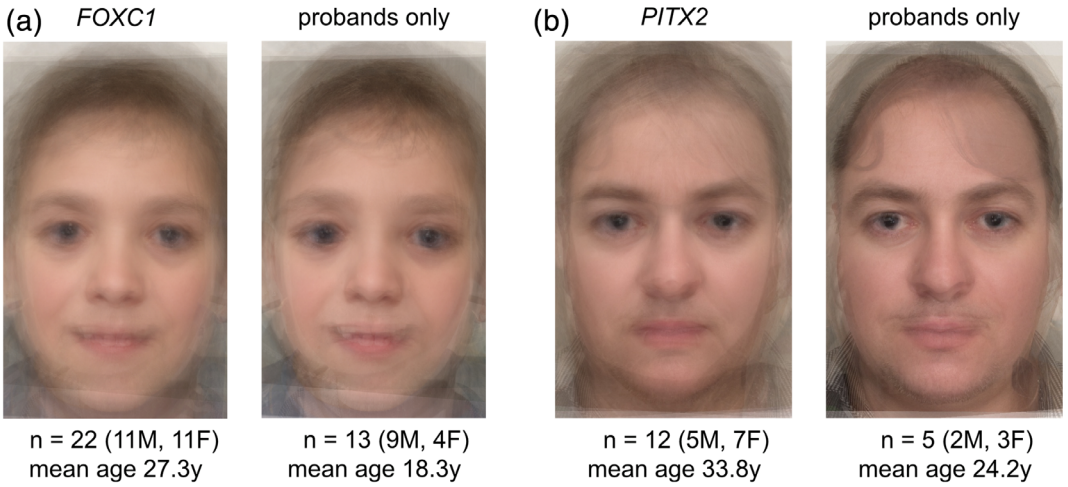

FIGURE 1 Face averaging of individuals with *FOXC1* or *PITX2* variants. (a) Individuals with *FOXC1* variants. (b) Individuals with *PITX2* variants. The "probands only" images were derived from individuals with photographs taken at the youngest age within each family. M; Male, F; Female

in reaching a proper clinical diagnosis. Although the presence of systemic features can facilitate differentiation between *FOXC1*- or *PITX2*-associated ARS (Souzeau et al., 2017), symptoms such as dental anomalies, hearing loss or growth delay may not manifest before an older age. Therefore, differences in facial dysmorphism may facilitate this differentiation prior to molecular testing. To the best of our knowledge, this is the first study to systematically assess the prevalence of facial features in individuals with *FOXC1* and *PITX2* variants, including whether there are any significant differences between both groups of individuals. Early descriptions of individuals with ARS have reported facial dysmorphism as a common feature (Alkemade, 1969; Rieger, 1941). However, studies following the identification of the *FOXC1* and *PITX2* genes have seldom reported on the facial appearance of affected individuals. Hypertelorism, maxillary hypoplasia and a broad nasal bridge seem to be the most commonly reported traits in *FOXC1*-associated ARS across different populations (D'Haene et al., 2011; Kawase et al., 2001; Kim et al., 2013; Micheal et al., 2016; Weisschuh et al., 2006), whereas maxillary hypoplasia and a thin upper lip are often reported in *PITX2*-associated ARS (D'Haene et al., 2011; Reis et al., 2012; Weisschuh et al., 2006). No study has systematically assessed affected individuals for the presence or absence of facial traits. In this study, 11 facial traits were assessed by five clinical geneticists for their presence or absence in a cohort of 34 individuals with ARS and a molecular diagnosis. Individuals with *FOXC1*-associated ARS were more likely to have hypertelorism/telecanthus and low-set ears compared to individuals with *PITX2*-associated ARS. Although not significant, maxillary hypoplasia was more prevalent in individuals with *FOXC1* variants and a thin upper lip was more prevalent in individuals with *PITX2* variants. These findings are consistent with previous isolated reports of facial

dysmorphism in *FOXC1*- or *PITX2*-associated ARS (D'Haene et al., 2011; Kawase et al., 2001; Kim et al., 2013; Reis et al., 2012).

Additionally, we analyzed the data including only one individual per family to account for facial traits that may be common between members of the same family. This analysis was done using the youngest individual in each family to support clinical diagnosis at the youngest possible age. Hypertelorism/telecanthus remained significantly more prevalent in individuals with *FOXC1* variants compared with *PITX2* variants. The presence of low-set ears was still more common in individuals with *FOXC1* variants but was not significantly different, potentially due to the smaller size of the cohort.

We previously reported that individuals with *FOXC1* variants are more likely to have no other systemic features than individuals with *PITX2* variants (Souzeau et al., 2017). Dental and umbilical anomalies are more commonly seen with *PITX2* variants whereas hearing loss is more commonly reported with *FOXC1* variants. These findings were confirmed in this cohort. Although the prevalence of heart defects was not significantly different between individuals with *FOXC1* and *PITX2* variants, *FOXC1* variants were associated with congenital heart defects whereas *PITX2* variants were associated with arrhythmogenesis. Testing for both genes is often performed for patients with ARS.Nevertheless differences in facial dysmorphism or systemic features can guide the prioritization of gene testing and the analysis of genetic results in the context of a well-characterized phenotype.

The phenotypic differences in craniofacial morphology that we observed might be related to the underlying developmental mechanisms regulated by *PITX2* and *FOXC1*. PITX2 protein plays a critical role in early development of structures in the front part of the eye, as well as the pituitary cells, teeth, heart, and abdominal organs (Franco et al., 2014). FOXC1 protein is a transcriptional partner of GLI2 highly expressed in undifferentiated mesenchyme and is thought to play a regulator role in chondrogenesis, initiating the ossification of cranial base bones important for craniofacial morphogenesis (Mya et al., 2018; Yoshida et al., 2015).

Limitations of this study included a small sample size with the majority of individuals of European ancestry. Additional studies will be needed to reproduce these findings in individuals of non-European ancestry. A subjective assessment of the facial traits was conducted to mimic the assessment that would be performed by geneticists in a real life clinic. Each trait was assessed by five experienced geneticists with consensus to be reached among four of them to address the subjective approach. ARS being a rare condition, we used clinical photographs to increase the cohort size. However, clinical photographs are not all to scale, therefore specific measurements for the different traits were not practicable. Additionally, skull X-rays would be needed to confirm the presence of hypertelorism which corresponds to an increased distance between the orbital cavities. Finally, facial traits change with age in some genetic syndromes and can become more or less characteristic as the individual grows. Our cohort was too small to analyze age-related changes and it remains to be seen whether the facial traits in *FOXC1*- or *PITX2*-associated ARS change with age.

In summary, our findings reveal a genotype–phenotype correlation within the spectrum of ARS-associated facial dysmorphism. These findings have implications for assessing affected individuals to reach clinical and molecular diagnoses, and for genetic counseling.

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AUTHOR CONTRIBUTIONS

Emmanuelle Souzeau: Conception and design, acquisition of data, analysis and interpretation of data, drafting the manuscript.

Owen M Siggs: Conception and design, analysis and interpretation of data, critically revised the manuscript.

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DATA AVAILABILITY STATEMENT

The ocular and systemic features that support the findings of this study are available in Supplementary Table 1.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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